

Endpoint dilution using an antibody to the adenoviral hexon protein may be used to quantitate virus production or infection efficiency of target cells (Armentano et al. (1995) Hum. Gene Ther. 6:1343-53.

In addition to containing the DNA sequences encoding one or more transgenes, the chimeric adenoviral vectors of the invention may contain any expression control sequences such as a promoter or enhancer, a polyadenylation element, and any other regulatory elements that may be used to modulate or increase expression, all of which are operably linked in order to allow expression of the transgene. The use of any expression control sequences, or regulatory elements, which facilitate expression of the transgene is within the scope of the invention. Such sequences or elements may be capable of generating tissue-specific expression or be susceptible to induction by exogenous agents or stimuli.

#### **Construction of Retroviral Vectors**

The antigen presenting cells described herein can also be genetically modified with retroviral vectors produced recombinantly by procedures already taught in the art. For example, WO 94/29438 describes the construction of retroviral packaging plasmids and packaging cell lines. As is apparent to the skilled artisan, the retroviral vectors useful in the methods of this invention are capable of infecting the cells described herein. The techniques used to construct vectors, and transfect and infect cells are widely practiced in the art. Examples of retroviral vectors are those derived from murine, avian or primate retroviruses. Retroviral vectors based on the Moloney murine leukemia virus (MoMLV) are the most commonly used because of the availability of retroviral variants that efficiently infect human cells. Other suitable vectors include those based on the Gibbon Ape Leukemia Virus (GALV) or HIV.

The range of host cells that may be infected by a retrovirus or retroviral vector is determined by the viral envelope protein. The recombinant virus can be used to infect virtually any other cell type recognized by the env protein provided by the packaging cell, resulting in the integration of the viral genome in the

transduced cell and the stable production of the foreign gene product. In general, murine ecotropic env of MoMLV allows infection of rodent cells, whereas amphotropic env allows infection of rodent, avian and some primate cells, including human cells. Amphotropic packaging cell lines for use with MoMLV systems are known in the art and commercially available and include, but are not limited to, PA12 and PA317. Miller et al. (1985) Mol. Cell. Biol. 5:431-437; Miller et al. (1986) Mol. Cell. Biol. 6:2895-2902; and Danos et al. (1988) Proc. Natl. Acad. Sci. USA 85:6460-6464. Xenotropic vector systems exist which also allow infection of human cells.

The host range of retroviral vectors has been altered by substituting the env protein of the base virus with that of a second virus. The resulting, "pseudotyped", virus has the host range of the virus donating the envelope protein and expressed by the packaging cell line. Recently, the G-glycoprotein from vesicular stomatitis virus (VSV-G) has been substituted for the MoMLV env protein. Burns et al. (1993) Proc. Natl. Acad. Sci USA 90:8033-8037; and PCT patent application WO 92/14829. Since infection is not dependent on a specific receptor, VSV-G pseudotyped vectors have a broad host range.

Usually, the vectors will contain at least two heterologous genes or gene sequences: (i) the therapeutic gene to be transferred; and (ii) a marker gene that enables tracking of infected cells.

The viral vector can be modified to incorporate chimeric envelope proteins or nonviral membrane proteins into retroviral particles to improve particle stability and expand the host range or to permit cell type-specific targeting during infection. The production of retroviral vectors that have altered host range is taught, for example, in WO 92/14829 and WO 93/14188. Retroviral vectors that can target specific cell types *in vivo* are also taught, for example, in Kasahara et al. (1994) Science 266:1373-1376. Kasahara et al. describe the construction of a Moloney leukemia virus (MoMLV) having a chimeric envelope protein consisting of human erythropoietin (EPO) fused with the viral envelope protein. This hybrid virus shows tissue tropism for human red blood progenitor cells that bear the

receptor for EPO, and is therefore useful in gene therapy of sickle cell anemia and thalassemia. Retroviral vectors capable of specifically targeting infection of cells are preferred for *in vivo* gene therapy.

5 The vector construct is introduced into a packaging cell line which will generate infectious virions. Packaging cell lines capable of generating high titers of replication-defective recombinant viruses are known in the art, see for example, WO 94/29438. Viral particles are harvested from the cell supernatant and purified for *in vivo* infection using methods known in the art such as by filtration of supernatants 48 hours post transfection. The viral titer is determined by infection  
10 of a constant number of appropriate cells (depending on the retrovirus) with titrations of viral supernatants. The transduction efficiency can be assayed 48 hours later by a variety of methods, including Southern blotting.

After viral transduction, the presence of the viral vector in the transduced cells or their progeny can be verified such as by PCR. PCR can be performed to  
15 detect the marker gene or other virally transduced sequences. Generally, periodic blood samples are taken and PCR conveniently performed using e.g. NeoR probes if the NeoR gene is used as marker. The presence of virally transduced sequences in bone marrow cells or mature hematopoietic cells is evidence of successful reconstitution by the transduced cells. PCR techniques and reagents are well  
20 known in the art, See, generally, PCR PROTOCOLS, A GUIDE TO METHODS AND APPLICATIONS. Innis, Gelfand, Sninsky & White, eds. (Academic Press, Inc., San Diego, 1990) and commercially available (Perkin-Elmer).

*In vitro/ex vivo*, exposure of human DCs to adenovirus (Ad) vector at a multiplicity of infection (MOI) of 500 for 16-24 h in a minimal volume of serum-free medium reliably gives rise to transgene expression in 90-100% of DCs. The  
25 efficiency of transduction of DCs or other APCs can be assessed by immunofluorescence using fluorescent antibodies specific for the tumor antigen being expressed (Kim et al., 1997). Alternatively, the antibodies can be conjugated to an enzyme (e.g. HRP) giving rise to a colored product upon reaction

with the substrate. The actual amount of antigen being expressed by the APCs can be evaluated by ELISA.

Transduced APCs can subsequently be administered to the host via an intravenous, subcutaneous, intranasal, intramuscular or intraperitoneal route of delivery.

*In vivo* transduction of DCs, or other APCs, can potentially be accomplished by administration of Ad (or other viral vectors) via different routes including intravenous, intramuscular, intranasal, intraperitoneal or cutaneous delivery. The preferred method is cutaneous delivery of Ad vector at multiple sites using a total dose of approximately  $1 \times 10^{10}$ - $1 \times 10^{12}$  i.u. Levels of *in vivo* transduction can be roughly assessed by co-staining with antibodies directed against APC marker(s) and the TAA being expressed. The staining procedure can be carried out on biopsy samples from the site of administration or on cells from draining lymph nodes or other organs where APCs (in particular DCs) may have migrated. The amount of TAA being expressed at the site of injection or in other organs where transduced APCs may have migrated can be evaluated by ELISA on tissue homogenates.

Although viral gene delivery is more efficient, DCs can also be transduced *in vitro/ex vivo* by non-viral gene delivery methods such as electroporation, calcium phosphate precipitation or cationic lipid/plasmid DNA complexes. Transduced APCs can subsequently be administered to the host via an intravenous, subcutaneous, intranasal, intramuscular or intraperitoneal route of delivery.

*In vivo* transduction of DCs, or other APCs, can potentially be accomplished by administration of cationic lipid/plasmid DNA complexes delivered via the intravenous, intramuscular, intranasal, intraperitoneal or cutaneous route of administration. Gene gun delivery or injection of naked plasmid DNA into the skin also leads to transduction of DCs. Intramuscular delivery of plasmid DNA may also be used for immunization.

The transduction efficiency and levels of transgene expression can be assessed as described above for viral vectors.

### Expansion of Immune Effector Cells

5           The present invention makes use of these APCs to stimulate production of an enriched population of antigen-specific immune effector cells. The antigen-specific immune effector cells are expanded at the expense of the APCs, which die in the culture. The process by which naïve immune effector cells become educated by other cells is described essentially in Coulie (1997) *Molec. Med.*  
10       Today 3:261-268.

          In a preferred embodiment, the antigen-specific immune effector cells are CTLs. In one aspect, the cytotoxic T cells are polyclonal T cells isolated from a site of cytotoxic T cell infiltration from a subject. Alternatively, such cells may be isolated from a site of cytotoxic T cell infiltration from two or more subjects or  
15       human patients, in which the subjects share an MHC halotype. In another embodiment, the CTLs may be two or more cytotoxic T cell lines. In yet another embodiment, the CTLs may be any combination of the foregoing.

          In a further aspect of the invention, the site of cytotoxic T cell infiltration is a tumor. The tumors from which cells or cell lines are obtained can be the same  
20       type of tumor in different individuals with a shared MHC halotype or different types of tumors from different subjects who share an MHC haplotype.

          The APCs prepared as described above are mixed with naïve immune effector cells. Preferably, the cells may be cultured in the presence of a cytokine, for example IL2. Because dendritic cells secrete potent immunostimulatory  
25       cytokines, such as IL2, it may not be necessary to add supplemental cytokines during the first and successive rounds of expansion. In any event, the culture conditions are such that the antigen-specific immune effector cells expand (*i.e.* proliferate) at a much higher rate than the APCs. Multiple infusions of APCs and optional cytokines can be performed to further expand the population of antigen-  
30       specific cells.

In one embodiment, the immune effector cells are T cells and are specific for tumor-specific antigens which are presented by the APCs.

### **Compositions**

5           This invention also provides compositions containing any of the above-mentioned proteins, muteins, polypeptides, nucleic acid molecules, vectors, cells antibodies and fragments thereof, and an acceptable solid or liquid carrier. When the compositions are used pharmaceutically, they are combined with a "pharmaceutically acceptable carrier" for diagnostic and therapeutic use. These  
10       compositions also can be used for the preparation of medicaments for the diagnosis and treatment of diseases such as cancer.

### **Tumor Protection in Animal Models**

Applicants are the first to establish that, based on the animal models  
15       described below, prevaccination with the compositions of this invention will prevent or delay onset of disease.

The murine B16 melanoma model was used. In this model, C57BL/6 mice were immunized with bone marrow-derived DCs transduced with an Ad vector encoding either human gp100 (Ad/hugp100) or mouse gp100 (Ad/mgpl00). Mice  
20       immunized against heterologous human gp100 developed a protective immune response and were resistant to a lethal subcutaneous challenge of B16 melanoma cells (syngeneic tumor cell line that expresses gp100). In contrast, mice immunized with homologous mouse gp100 failed to mount a protective immune response against B16 melanoma cells and developed tumors at the site of B16 cell  
25       injection. This finding illustrates the difficulty in breaking tolerance against a self antigen (mouse gp100). The corresponding heterologous antigen from a different species (human gp100), however, is likely to contain several Class I and Class II-associated epitopes that will be recognized as foreign and elicit CD8<sup>+</sup> and CD4<sup>+</sup> T cell responses, respectively. The induction of cross-reactive CTLs that recognize

both the heterologous and homologous self-antigen can then lead to lysis of host tumor cells.

Unfortunately, this type of animal model cannot be used to test the efficacy of modified or heterologous tumor antigens being considered for use in humans since mice and humans recognize different epitopes, primarily as a result of differences in their MHC molecules. It may be possible, however, to use the allogeneic human peripheral blood lymphocyte - severe combined immunodeficiency mouse (Hu-PBL-SCID) model. SCID mice lack mature B and T lymphocytes and can be reconstituted with human PBLs. It may be possible to immunize such mice with test antigen to induce a response in adoptively transferred human PBLs and evaluate protection against challenge with a human tumor cell line (Mosier et al., 1988; Parney et al., 1997; Albert et al., 1997).

Another possibility is immunization of HLA-A2.1 transgenic mice to reproduce the immune reactivity of HLA-A2 individuals.

#### **Adoptive Immunotherapy and Vaccines**

The expanded populations of antigen-specific immune effector cells of the present invention find use in adoptive immunotherapy regimes and as vaccines.

Adoptive immunotherapy methods involve, in one aspect, administering to a subject a substantially pure population of educated, antigen-specific immune effector cells made by culturing naïve immune effector cells with APCs as described above. Preferably, the APCs are dendritic cells.

In one embodiment, the adoptive immunotherapy methods described herein are autologous. In this case, the APCs are made using parental cells isolated from a single subject. The expanded population also employs T cells isolated from that subject. Finally, the expanded population of antigen-specific cells is administered to the same patient.

In another embodiment, the adoptive immunotherapy methods are allogeneic. Here, cells from two or more patients are used to generate the APCs, and stimulate production of the immune effector cells. For instance, cells from

other healthy or diseased subjects can be used to generate antigen-specific cells in instances where it is not possible to obtain autologous T cells and/or dendritic cells from the subject providing the biopsy. The expanded population can be administered to any one of the subjects from whom cells were isolated, or to  
5 another subject entirely.

In a further embodiment, APCs or immune effector cells are administered with an effective amount of a stimulatory cytokine, such as IL-2 or a co-stimulatory molecule.

The agents identified herein as effective for their intended purpose can be administered to subjects or individuals susceptible to or at risk of developing a  
10 disease, such as cancer. When the agent is administered to a subject such as a mouse, a rat or a human patient, the agent can be added to a pharmaceutically acceptable carrier and systemically or topically administered to the subject. To determine patients that can be beneficially treated, a tumor regression can be  
15 assayed. Therapeutic amounts can be empirically determined and will vary with the pathology being treated, the subject being treated and the efficacy and toxicity of the therapy. When delivered to an animal, the method is useful to further confirm efficacy of the agent. As an example of an animal model, groups of nude mice (Balb/c NCR nu/nu female, Simonsen, Gilroy, CA) are each subcutaneously  
20 inoculated with about  $10^5$  to about  $10^9$  hyperproliferative, cancer or target cells as defined herein. When the tumor is established, the agent is administered, for example, by subcutaneous injection around the tumor. Tumor measurements to determine reduction of tumor size are made in two dimensions using vernier calipers twice a week. Other animal models may also be employed as  
25 appropriate.

Administration *in vivo* can be effected in one dose, continuously or intermittently throughout the course of treatment. Methods of determining the most effective means and dosage of administration are well known to those of skill in the art and will vary with the composition used for therapy, the purpose of  
30 the therapy, the target cell being treated, and the subject being treated. Single or

multiple administrations can be carried out with the dose level and pattern being selected by the treating physician. Suitable dosage formulations and methods of administering the agents can be found below.

5 The agents and compositions of the present invention can be used in the manufacture of medicaments and for the treatment of humans and other animals by administration in accordance with conventional procedures, such as an active ingredient in pharmaceutical compositions.

10 More particularly, an agent of the present invention also referred to herein as the active ingredient, may be administered for therapy by any suitable route including nasal, topical (including transdermal, aerosol, buccal and sublingual), parenteral (including subcutaneous, intramuscular, intravenous and intradermal) and pulmonary. It will also be appreciated that the preferred route will vary with the condition and age of the recipient, and the disease being treated.

15 The compositions and methods described herein are particularly useful in providing or inducing a prophylactic immune response in an animal. Animals in a pre-disease state or in a disease free interval, i.e., having or pre-disposed to a condition subject to immune surveillance, are most suitably treated by the methods and compositions described herein. Such conditions involve the activation of an immune response in a diseased state or period.

20

#### Example

25 Dendritic cells were derived from peripheral blood of a HLA-A2+ human donor using standard GM-CSF/IL-4 culture technique. After six days, the cells were infected with an adenovirus (serotype 2) construct encoding human gp 100. At T=7 days, they were restimulated with  $5 \times 10^5$  infected autologous DCs (from frozen stocks) and given 50 U/ml rhIL-2. At T=14 days, the cultures were treated with leucyl-leucyl-methyl ester in order to eliminate NK cell activity. Immediately after treatment, the cells were washed thoroughly and replated along  
30 with  $1 \times 10^7$  mitomycin C-treated autologous PBMC as feeders. At T=21 days,

cells were split and replated at  $5 \times 10^5$  cells/ml in Iscoves/10% human AB serum/1000U rhIL-2/ml. At T=26 days, the CTLs raised against gp100 were tested in  $^{51}\text{Cr}$ -release assay using peptide pulsed T2 cells as targets. Effector CTLs were TIL 1520 which specifically recognize an HLA-A-A2 restricted epitope of the wild-type human gp100 protein. Figure 5 shows the results of this assay. There is epitope-specific recognition that was not present prior to education of the T cells with the infected DCs.

It is to be understood that while the invention has been described in conjunction with the above embodiments, that the foregoing description and the following examples are intended to illustrate and not limit the scope of the invention. For example, any of the above-noted compositions and/or methods can be combined with known therapies or compositions. Other aspects, advantages and modifications within the scope of the invention will be apparent to those skilled in the art to which the invention pertains.

CLAIMS

1. A method of inducing a prophylactic immune response to a self-antigen in a subject, comprising administering to the subject an effective amount  
5 of the antigen or an altered form of the antigen.

2. The method of claim 1, wherein the antigen is administered as a polynucleotide coding for the self-antigen.

10 3. The method of claim 2, wherein the polynucleotide is delivered as naked DNA.

4. The method of claim 2, wherein the polynucleotide is delivered in a gene delivery vehicle.  
15

5. The method of claim 1, further comprising administering an effective amount of an immunostimulatory agent to the subject.

20 6. The method of claim 5, wherein the immunostimulatory agent is administered as a polynucleotide coding for the immunostimulatory agent.

7. The method of claim 1, wherein the antigen is administered in an antigen presenting cell.

25 8. The method of claim 7, wherein the antigen presenting cell has been genetically modified by insertion of a polynucleotide coding for the antigen.

9. The method of claim 7, wherein the antigen presenting cell is a foster antigen presenting cell, a hybrid antigen presenting cell, or a pulsed antigen  
30 presenting cell.

10. The method of claim 7, wherein the antigen presenting cell is a dendritic cell.

5 11. The method of claim 7, further comprising administering an effective amount of an immunostimulatory agent to the subject.

12. The method of claim 11, wherein the immunostimulatory agent is administered as a polynucleotide coding for the immunostimulatory agent.

10

13. The method of claim 1 or 7, wherein the self-antigen is a tumor associated antigen (TAA).

14. A method of providing a prophylactic immune response to a self-antigen in a subject, comprising administering to the subject an effective amount of educated immune effector cells, educated to specifically recognize and lyse cells expressing the self-antigen or an altered form of the self-antigen.

15

15. The method of claim 14, wherein the immune effector cells have been produced by stimulating naïve immune effector cells with antigen presenting cells that present the antigen or an altered self-antigen to the naïve immune effector cells.

20

16. The method of claim 14, wherein the educated immune effector cells are produced *ex vivo*.

25

17. The method of claim 14, wherein the educated immune effector cells are produced *in vivo*.

18. The method of claim 14, further comprising administering an effective amount of an immunostimulatory agent.

5 19. The method of claim 18, wherein the immunostimulatory agent is administered as a polynucleotide coding for the immunostimulatory agent.

20. The method of claim 1 or 14, wherein the subject is characterized as being in a disease-free state but genetically predisposed to a condition subject to immune surveillance.

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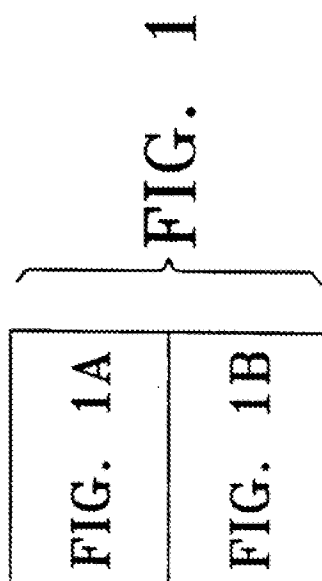
21. The method of claim 20, wherein the condition is associated with the presence of the HER-2/neu gene in the subject.

22. The method of claim 1 or 14, wherein the subject is characterized as being in a disease free interval of a condition subject to immune surveillance.

15

23. The method of claim 22, wherein the condition is melanoma.

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HUMAN	1	MDLVLKRCLL	HLAVIGALLA	VGATKVPRNQ	DWLGVSQRLR	TKAWNRLQLYP
MOUSE		--*G-QR-SF-	PLV-LSA---	---LEGS---	-----P---V	--T-----
	51	EWTEAQRDC	WRGCGVSLKV	SNDGPTLIGA	NASFSLALNF	PGSQKVLPDG
		----V-GSN-	-----R-	I----- --	-----H-	-----
	101	QVIWVNNTII	NGSQVWGGQP	VYPQETDDAC	IFPDGGPCPS	GSWSQKRFSV
		----A----	-----	---P----	V-----	-PKPP----
	151	YVWKIWGQYW	QVLGCPVSGL	SIGTGRAMLG	THIMEVTVYH	RRGSRSYVPL
		-----K--	-----R-	--A--H-K--	-----	----Q----
	201	AHSSSAFTIT	DQVPFVSVS	QLRALDGGNK	HFLRNQPLTF	ALQLHDPSCY
		--A--T----	-----	--Q-----ET-	-----H--I-	-----
	251	LAEADLSYTW	DFGDSSGTLI	SRALVVTHTY	LEPGPVTAQV	VLQAAIPLTS
		-----GT----	-----D----	-----S-S-	-----	-----V-

FIG. 1A

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301 CGSSPVPGIT DGHRTAEAP NITAGQVPTT EVVGTTPGQA PTAEPSGITS
    ----- --YM----- G--SR-GT-- -----M--TQ-----V

351 VQVPTTEVIS TAPVQMPTAE STGMTPEKVP VSEVMGTILA EMSTPEATGM
    --M-----TA -TSE--L--* ***** **A-ID----- -V--T-G--T

401 TPAEVSIVVL SGTIAAQVTT TEWVETTARE LPIPEPEGPD ASSIMSTESI
    --T*****P -----V--A-- --***** **----- --PLLP-Q-S

451 TGGLGPLLDG TATRLVKRQ VPLDCVLYRY GSFSVTLDIV QGIESAEILQ
    ---IS-----D -D-IM----- -----LA-----

501 AVPSGEGDAF ELTVSCQGGL PKEACMEISS PGCQPPAQR L CQVLPSPAC
    ---FS----- -----D----- -----S-P---D-

551 QLVLHQILKG GSGTYCLNVS LADTNSLAVV STQLIMPQE AGLGQVPLIV
    -----V----- -----A-----A -----VV---D G-----A--L-

601 GILLVLMVV LASLIYRRRL MKQDFSPQL PHSSHVLR L PRIFCSCPIG
    -----V----- -----H-H-- K--G*--S-M --G-T----- -PV-RARGL-

651 ENSPLLGGQ VX
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FIG. 1B

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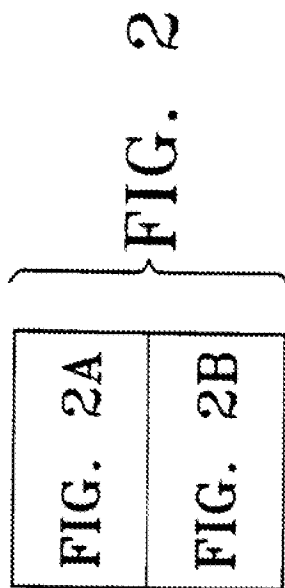


FIG. 2A

A	mMART	10 20 30 40 50	ATGCCCCAAGAAGACATTCACTT-----TGGTTATCCCAAGGCGCACAGACGCTCC	30 40 50
	hMART	10 20 30 40 50 60	ATGCCCCAAGAAGATGCTCACTTTCATCTATGTTACCCCAAGAAGGCGCACGGCCACTCT	30 40 50 60
	mMART	60 70 80 90 100 110	TATGTCACCTGCTGAAGAGCGCGCAGGGATCGGCATCCTGATCGTGGTCCCTGGGATTGCT	90 100 110
	hMART	60 70 80 90 100 110 120	TACACCACGGCTGAAGAGCGCGCTGGGATCGGCATCCTGACAGTGTATCCCTGGGAGTCTTA	90 100 110 120
	mMART	120 130 140 150 160 170	CTGCTTATCGGCTGCTGGTACTGTAGAAGACGAAGTGGATACAGAACCTTGTATGGACAAA	130 140 150 160 170
	hMART	120 130 140 150 160 170 180	CTGCTCATCGGCTGTGGTATTGTAGAAGACGAATGGATACAGAGCCTTGTATGGATAAA	130 140 150 160 170 180
	mMART	180 190 200 210 220 230	AGCGCTCATATTGGTATTCAAAAACCTCAAGGGAAGATGCTCATGTGAGAGCCCTGAT	190 200 210 220 230
	hMART	180 190 200 210 220 230 240	AGCTTTCATGTTGGCACTCAATGTGCCCTTAACAAGAAGATGCCCAACAAGAAGGGTTTGT	190 200 210 220 230 240

[illegible]

FIG. 2B

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FIG. 3

FIG. 3A

FIG. 3B

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1  AGCAGACAGAGGACTCTCATTAAGGAAGG  TGTCCTGTGCCCTTGACCCCTACAAGATGCCA
MetPro

120  ACGGCTGAAGAGGCCGCTGGGATCGGCATC  CTGACAGTGAATCCITGGGAGTCTTACTGCTC
23  ThrAlaGluGluAlaAlaGlyIleGlyIle  LeuThrValIleLeuGlyValLeuLeuLeu

240  CATGTTGGCACITCAATGTGCCITTAACAAGA  AGATGCCCCACAAGAAGGTTTGTATCATCGG
63  HisValGlyThrGlnCysAlaLeuThrArg  ArgCysProGlnGluGlyPheAspHisArg

360  GCATTATCAGAAACTCTCTGCAGAACAGTCA  CCACCACCITATTACCTTAAGAGCCACGG
103  AlaTyrGluLysLeuSerAlaGluGlnSer  ProProProTyrSerPro

480  ATCTAATGTTCTCCTTTGGAAATGGGTAGG  AAAAATGCAAGCCATCCTCTAATAATAAGTC
600  TATTAAATTGGCAAAACTCCATCAATAAAT  GTTGCAATGCATGATACTATCTGTGCCAGA
720  GGGGCCATCCAAATTTCTCTTTTACTTGAAAT  TTGGCTAATAACAACAACCTAGTCAGGTTTTCG
840  GATACITTTACAGGTTAAGACAAAGGGTTG  ACTGGCCTATTATCTGATCAAGAACAATGT
960  CTATAGCTCTTTTTTTTGGAGATGGAGTTT  CGCTTTTGTGGCCAGGCTGGAGTGCATG
1080  CCTCCTGAGTAGCTGGGATTACAGGCGGCG  GCCACTATGCCCTGACTAATTTGTAGTTT
1200  TCTGCCCCGCTCAGCCTCCCAAAGTCTGG  AATTACAGGCGTGAAGCCACCCCTGGCT
1320  AATGCTATTCTAACTAATGACAAAGTATTT  CTACTAAACCAGAAATTGGTAGAAGGATTT
1440  TACCTATGGCAATTAGCTCTCTTGGGTTC  CCAATCCCTCTCACAGAATGTGCCAGAAG

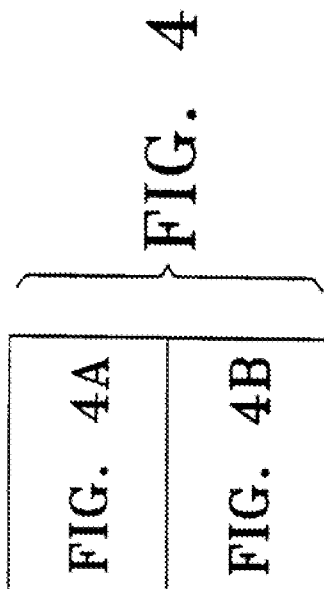
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FIG. 3A

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AGAGAAAGATGCTCAGCTTCATCTATGGTTAC	CCCAAGAAGGGGCACGGCCACCTCTACACC	119
ArgGluAspAlaHisPheIleTyrGlyTyr	ProLysLysGlyHisGlyHisSerTyrThr	22
ATCGGCTGTGGTATTGTAGAACGACGAAAT	GGATACAGAGCCCTTGATGGATAAAAGTCIT	239
<u>IleGlyCysTrpTyrCysArgArgArgAsn</u>	GlyTyrArgAlaLeuMetAspLysSerLeu	62
GACAGCAAAGTGTCCTCTTCAAGAGAAAAAC	TGTGAACCTGTGGTTCCCAATGCTCCACCT	359
AspSerLysValSerLeuGlnGluLysAsn	CysGluProValValProAsnAlaProPro	102
AGACACCTGAGACATGCTGAAATTATTCT	CTCACACCTTTTGGCTTGAATTTAATACAGAC	479
		118
AGTGTTAAAAATTTIAGTAGGTCGGCTAGCA	GTACTAATCAATGTGAGGAAATGATGAGAAA	599
GGTAAATGTTAGTAAATCCATGGTGTTATTT	TCTGAGAGACAGCAATTCAAGTGGGTATTCT	719
AACCTTGACCGACATGAACCTGTACACAGAA	TTGTTCCAGTACTATGGAGTGCCTCACAAAG	839
CAGCAATGTCCTTTGTGCTCTAAAATTCT	ATTATACTACAATAATATATTGTAAAGATC	959
GCGGATCTTGGCTCACCATAAACCTCCGCC	TCCCAGGTTCAAGCAATTCCTCCCTTAG	1079
AGTAGAGACCGGGTTCTCCATGTGGTCA	GGCTGGTCTCAAACTCCTGACCCTCAGGTGA	1199
GGATCCCTATATCTTAGGTAAGACATAAATAC	GCAGTCTAATTACATTTACACTTCAAGGCTC	1319
AAATAAGTAAAGCTACTATGTACTGCCCIT	AGTGTGATGCTGTGCTGCTTAAATG	1439
AAATCATAAAGGATCAGAGATTCTGAAAAA	AAAAAAAATAAAAAATAAAAAATAAAAAA	1559

FIG. 3B



MOUSE TRP2

1 MCLVGWGLL GCLGCGILLR ARAQFPRVCM TLDGVLNKEC CPPLGPEATN  
 51 ICGFLEGRGQ CAEVQTDTRP WSGPYILRNQ DDREQWPRKF FNRICKCTGN  
 101 FAGYNCGGCK FGWTGPDCNR KKPAILRRNI HSLTAQEREQ FLGALDLAKK  
 151 SIHPDYVITT QHWLGLLGN GTQPQIANCE VYDFFVWLHY YSVRDILLGP  
 201 GRPYKAIDFS HQGPAFVTWH RYHLLWLERE LQRLTGNESF ALPYWNFATG  
 251 KNECDVCTDD WLGAARQDDP TLISRNSRFS TWEIVCDSD DYNRRVTLCN  
 301 GTYEGLLRRN KVGRNNEKLP TLKNVQDCLS LQKFDSPFF QNSTFSFRNA  
 351 LEGFDKADGT LDSQVMNLHN LAHSFLNGTN ALPHSAANDP VFVVLHSFTD  
 401 AIFDEWLKRN NPSTDAWPQE LAPIGHNRMV NMVPFFPPVT NEELFLTAEQ  
 451 LGYNYAVDLS EEEAPVWSTT LGVVIGILGA FVLLGLLAF LQYRRLRKGY  
 501 APLMETGLSS KRYTEEA

FIG. 4A

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## HUMAN TRP2

1 MSPLWWGFLI SCLGCKILPG AQGFPRVCM TVDSLVNKEC CPRLGAESAN  
51 VCGSQQGRGQ CTEVRADTRP WSGPYILRNQ DDRELWPRKF FHRTCKCIGN  
101 FAGYNCGDCK FGWTGPNCEK KKPPVIRQNI HSLSPQEREQ FLGALDLAKK  
151 RVHPDYVITT QHWLGLLGN GTQPQFANCS VYDFFVWLHY YSVRDTLLGP  
201 GRPYRAIDFS HQGPAFVTVH RYHLLCLERD LQRLIGNESF ALPYWNFATG  
251 RNECDVCTDQ LFGAARPDDP TLISRSRFS SWETVCDSD DYNHLVTLCN  
301 GTYEGLLRN QMGRNSMKLP TLKDIRDCLS LQKFDNPPFF QNSTFSFRNA  
351 LEGFDKADGT LDSQVMSLHN LVHSFLNGTN ALPHSAANDP IFVVLHSFTD  
401 AIFDEWMKRF NPPADAWPQE LAPIGHNRMV NMVPFFPPVT NEELFLTSDQ  
451 LGYSYAIDL PVSVEETPGWP TLLVVMGTL VALVGLFVLL AFLQYRRLRK  
501 GYTPLMETHL SSKRYTEEA

FIG. 4B

9/9

Assay of CTLs Generated From  
Normal Donor PBL With Ad-GP100-  
Infected Dendritic Cells  
(Targets=T2 Cells Pulsed with GP100-  
F9 Peptide)

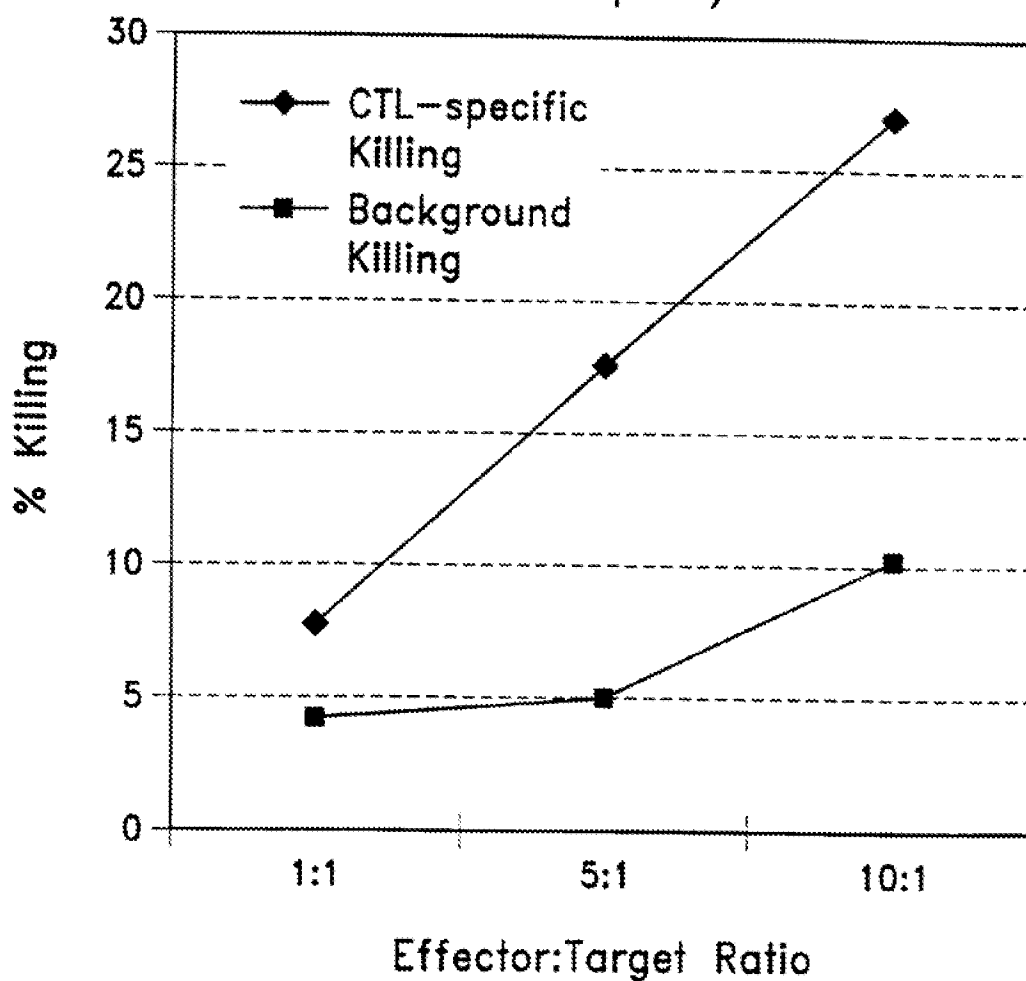


FIG. 5

## SEQUENCE LISTING

<110> Nicolette, Charles A.  
Genzyme Corporation

<120> COMPOSITIONS AND METHODS FOR ANTIGEN-SPECIFIC  
VACCINATION

<130> 159792001040

<140> Unassigned

<141> 1999-03-19

<150> 60/078,890

<151> 1998-03-20

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 His Arg Asp Ser Lys Val Ser Leu Gln Glu Lys Asn Cys Glu Pro Val  
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&lt;400&gt; 17

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Arg Ala Asp Thr Arg Pro Trp Ser Gly Pro Tyr Ile Leu Arg Asn Gln
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## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US99/06034

<b>A. CLASSIFICATION OF SUBJECT MATTER</b>		
IPC(6) : Please See Extra Sheet.		
US CL : Please See Extra Sheet.		
According to International Patent Classification (IPC) or to both national classification and IPC		
<b>B. FIELDS SEARCHED</b>		
Minimum documentation searched (classification system followed by classification symbols)		
U.S. : 424/184.1, 277.1, 287.1, 93.2, 93.71; 435/372, 373; 514/2, 44; 530/350, 806, 827, 828; 536/23.1, 23.5		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)		
DIALOG medicine index, APS, WEST		
<b>C. DOCUMENTS CONSIDERED TO BE RELEVANT</b>		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A, P	LIU, M. Transfected human dendritic cells as cancer vaccines. Nat. Biotech. April 1988, Vol. 16, pages 335-336, see entire document.	1-23
A	DONNELLY, J.J. et al. DNA vaccines. Ann. Rev. Immunol. 1997, Vol. 15, pages 617-648, see entire document.	1-23
A, P	PARDOLL, D.M. Cancer vaccines. Nature Medicine. May 1998, Vol. 4, No.5(suppl.), pages 525-531, see entire document.	1-23
<input checked="" type="checkbox"/> Further documents are listed in the continuation of Box C. <input type="checkbox"/> See patent family annex.		
* Special categories of cited documents:	*T*	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
*A* document defining the general state of the art which is not considered to be of particular relevance	*X*	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
*E* earlier document published on or after the international filing date	*Y*	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
*L* document which may throw doubt on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*G*	document member of the same patent family
*O* document referring to an oral disclosure, use, exhibition or other means		
*P* document published prior to the international filing date but later than the priority date claimed		
Date of the actual completion of the international search	Date of mailing of the international search report	
29 JUNE 1999	03 AUG 1999	
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	Authorized officer <i>D. Lawrence</i> F PIERRE VANDERVEGT	
Facsimile No. (703) 305-3230	Telephone No. (703) 308-0196	

## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US99/06034

## C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	BOCZKOWSKI, D. et al. Dendritic cells pulsed with RNA are potent antigen-presenting cells in vitro and in vivo. J. Exp. Med. 01 August 1996, Vol. 184, No. 2, pages 465-472, see entire document, abstract in particular.	1-3, 7-10, 14-17, 20, 22
X	US 5,679,647 A (CARSON et al) 21 October 1997, see entire document.	1-3, 13, 20-23
X, P ---- Y, P	US 5,844,075 A (KAWAKAMI et al) 01 December 1998, see entire document.	1, 13, 20, 22-23 ---- 2-12, 14-19, 21

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US99/06034

## A. CLASSIFICATION OF SUBJECT MATTER:

IPC (6):

A01N 37/18, 43/04, 63/00; A61K 31/70, 35/12, 35/36, 39/00; C12N 5/02, 5/06; C07K 1/00; C07H 21/02, 21/04

## A. CLASSIFICATION OF SUBJECT MATTER:

US CL :

424/184.1, 277.1, 287.1, 93.2, 93.71; 435/372, 373; 514/2, 44; 530/350, 806, 827, 828; 536/23.1, 23.5